ENUMERATIVE STUDIES ON *TRYPANOSOMA GAMBIENSE* AND *TRYPANOSOMA RHODESIENSE* IN RATS, GUINEA-PIGS, AND RABBITS; PERIODIC VARIATIONS DISCLOSED*

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(*Received for publication* 15 *October*, 1910)

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INTRODUCTION

The results recorded in this memoir were obtained chiefly from investigations on animals—rats, guinea-pigs and rabbits—sub-inoculated from a case of Rhodesian sleeping sickness recently studied by Major R. Ross and Dr. D. Thomson in the Royal Southern Hospital, Liverpool.

The investigations were undertaken at the suggestion of Major Ross, under funds allotted by the Tropical Diseases Research

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*An abstract of this paper was read before the Royal Society on December 8, 1910, and published in Proc. Roy. Soc., B. Vol. LXXXIII. pp. 206-211.

Committee in the case of one of us (H.B.F.), and under the Sir Edwin Durning-Lawrence Fund in the case of the other (J.G.T.). The researches on animals were necessary in order to determine whether a regular periodic increase in the parasites in the peripheral circulation occurred, similar to that found by R. Ross and D. Thomson* (1910) in the case of Rhodesian sleeping sickness. Again, it was necessary to determine whether this numerical cyclical development had a definite significance in the life-history of the parasite, which it would be necessary to consider in the case of treatment of trypanosomiasis by drugs or other methods. Our work was done in the Liverpool School of Tropical Medicine.

The trypanosome from the patient suffering from sleeping sickness contracted in Rhodesia was found by Stephens and Fantham (1910)† to exhibit a marked morphological peculiarity in the presence of a posterior nucleus in some of the stout forms, and for this parasite the name *T. rhodesiense* was suggested by them We have also used an old laboratory strain of *T. gambiense.*

OUTLINE OF THE METHODS USED

In all cases a definite number of trypanosomes was inoculated. The inoculation was made subcutaneously in rats, intraperitoneally in guinea-pigs and rabbits.

The peripheral blood of these animals was then examined daily, at the same hour, that is to say, at regular intervals of twenty-four hours.

The technique employed was that elaborated by R. Ross and D. Thomson (1910) in their study of the patient W.A., namely, that a known quantity of blood (one cubic millimetre divided into quarters) was taken and the parasites therein very carefully counted. In counting the thick films an Ehrlich eye-piece was used, after the films had been dehaemoglobinised, fixed with absolute alcohol and stained by the Romanowsky method.

When the parasites became very numerous it was necessary to spread the quarter cubic millimetre of blood over a larger square area than usual, and to use a very small diaphragm in the eye-piece. The parasites were then counted in three or four bands across the film, which had been previously examined in order to ascertain an even distribution of the trypanosomes therein—though this led to certain unavoidable errors (see R. Ross and D. Thomson on 'Enumerative Studies on Malarial Fever'*). It will be obvious that the counts entailed much labour, especially when the parasites were numerous.

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* Proc. Roy. Soc., B, Vol. LXXXII. pp. 411-415. and Vol. LXXXIII. pp. 187-205.
† Proc. Roy. Soc., B. Vol. LXXXIII. pp. 28-33.

We have endeavoured to estimate the number of divisions of the trypanosomes during the first twenty-four hours of their appearance in the peripheral blood of the host. (See pp. 433-4.)

ENUMERATIONS IN RATS

We now give a series of tables of enumerations in rats inoculated with (*a*) *T. rhodesiense* and (*b*) *T. gambiense.*

In the case of both strains it was found that the rats tended to fall into one of two main categories exhibiting either (1) a periodic increase and decrease in the number of parasites in the peripheral blood (see Chart 1), or (2) a more or less continuous rise in the numbers of parasites until the animal died (see Chart 2). (Cf. Moore and Breinl†.)

We therefore classify the animals inoculated with each strain into two groups:—

(*a*) *Trypanosoma rhodesiense*

I. RATS EXHIBITING PERIODIC VARIATION

RAT 1.—Piebald ♂, weight 187 grams. Dose of inoculation one million Trypanosomes. (H.B.F.)

Days	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	6	48	9,860	60,000	84,640
Temp.[1]	30	32	44	64	64	50	50
Day	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Parasites per c.mm.	7,760	24,000	20,480	63,800	32,000	21,760	89,600
Temp.[1]	28	10	2	30	22	0	10

[1] The temperature is expressed according to the following Haematothermic scale, as in Major Ross's recent papers on 'Malaria' and 'Trypanosomiasis':—Temp. = (F − 95) × 10, where F is the temperature in degrees Fahrenheit, recorded with a clinical thermometer, per rectum.

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* Proc. Roy. Soc., B, Vol. LXXXIII, p. 161, and Ann. Trop. Med. and Parasitol. (1910). Vol. IV, p. 268.

† Ann. Trop. Med. and Parasitology (1907). Vol. I. p. 448.

The graph of the number of parasites of this animal is represented in Chart 1.

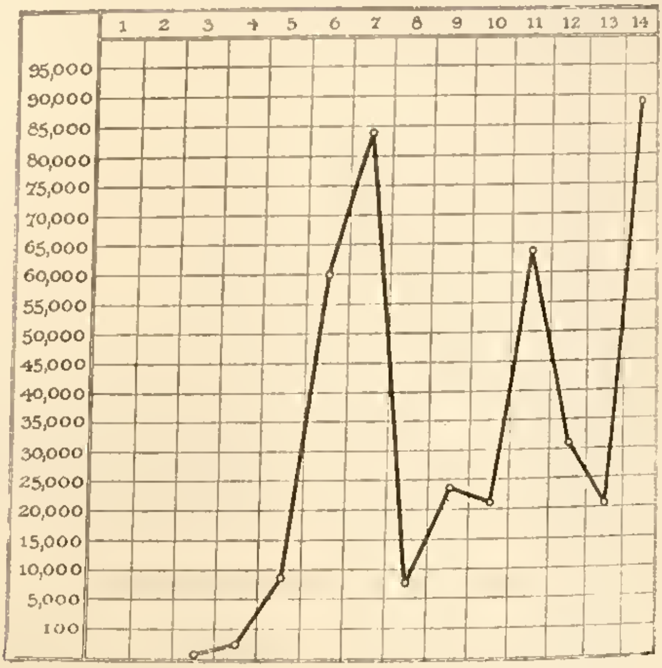

Chart 1.—Graph showing Daily Counts of the Parasites per c.mm. in the Peripheral Blood of a Rat inoculated with *T. rhodesiense*. Periodic Variation.

The numbers of the parasites are indicated along the vertical line, and the duration of infection in days along the horizontal line.

(See record of Rat 1.)

Rat 2.—White ♂, weight 108 grams. Dose 350,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	68	18,240	44,120	32,080	22,400
Temp.	32	24	20	40	56	38	22
Day	8	9	10	—	—	—	—
Parasites per c.mm.	12,860	126,000	61,840	—	—	—	—
Temp.	20	25	4	—	—	—	—

Rat 3.—White ♀, weight 225 grams. Dose: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	12	3,480	58,240	16,000	1,840	6,400
Temp.	20	20	14	86	106	40	15
Day	8	9	—	—	—	—	—
Parasites per c.mm.	52,000	42,400	—	—	—	—	—
Temp.	15	0	—	—	—	—	—

Rat 4.—Piebald ♂, weight 220 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	8	1,960	11,520	28,800
Temp.	40	53	50	50	34	36	74
Day	8	9	10	11	12	13	—
Parasites per c.mm.	56,320	84,000	50,000	39,200	160,000	100,400	—
Temp.	50	48	38	40	47	20	—

Rat 5.—White ♀, weight 120 grams. Dose: 750,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	896	3,280	32,560	51,600
Temp.	38	36	38	50	40	52	48
Day	8	9	10	11	—	—	—
Parasites per c.mm.	64,800	22,800	16,800	6,160	—	—	—
Temp.	44	22	20	10	—	—	—

Rat 6.—Piebald ♂, weight 100 grams. Dose: one million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	32	8,400	52,728	67,760
Temp.	40	40	46	56	60	50	14

Day	8	9	—	—	—	—	—
Parasites per c.mm.	96,000	40,656	—	—	—	—	—
Temp.	30	54	—	—	—	—	—

Rat 7.—Piebald ♂, weight 189 grams. Dose: 30,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	336	6,572	23,888	80,640	40,600
Temp.	38	40	70	72	66	70	44

Day	8	9	—	—	—	—	—
Parasites per c.mm.	30,250	150,000	—	—	—	—	—
Temp.	42	5	—	—	—	—	—

Rat 8.—White ♀, weight 195 grams. Dose: 100,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	100	0	896	60,000
Temp.	40	42	40	40	60	64	74

Day	8	9	10	11	12	13	—
Parasites per c.mm.	81,000	9,200	1,960	7,840	23,040	83,200	—
Temp.	55	56	70	60	68	52	—

Rat 9.—Piebald ♂, weight 100 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	20	4,672	8,040	70,400
Temp.				30	40	50	54

Day	8	9	10	11	12	—	—
Parasites per c.mm.	32,400	3,360	29,880	88,800	113,520	—	—
Temp.	58	46	42	40	35	—	—

Rat 10.—Piebald ♂, weight 150 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	12	3,620	8,400	2,520	7,280
Temp.	36	34	42	50	42	66	48	38

Day	9	10	11	12	13	14	15
Parasites per c.mm.	12,000	6,200	7,040	11,000	80,000	18,432	120,000
Temp.	46	34	22	40	44	24	20

Note slow rise in numbers of parasites.

Rat 11.—White ♀, weight 148 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	316	1,920	8,800	11,200
Temp.						54	66

Rat 11—continued.

Day	8	9	10	11	12	13	14
Parasites per c.mm.	2,580	1,040	52	1,032	2,348	26,400	54,000
Temp.	66	52	50				

Day	15	16	17	18	—	—	—
Parasites per c.mm.	7,360	12,120	31,460	85,460	—	—	—
Temp.					—	—	—

Note the slow rise, and the long period between the crests of the first waves, due probably to the great resistance of the animal.

Rat 12.—White ♀, weight 138 grams. Dose: 600,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	16	3,620	13,740
Temp.	34	42	54	44	54	74	66

Day	8	9	10	11	12	—	—
Parasites per c.mm.	34,272	6,048	3,640	24,360	21,600	—	—
Temp.	64	60	40	63	46	—	—

This animal was pregnant, and gave birth to five young rats on the tenth day.

Rat 13.—White, weight 140 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	4	84	8,928

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	0	0	560	0	7,004	1,408	2,856

Rat 13—continued.

Day	15	16	—	—	—	—	—
Number of Trypanosomes per c.mm.	118,272	162,288	—	—	—	—	—

This animal was pregnant, and gave birth to eight young rats on ninth day.

Rat 14.—Piebald, weight 127 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	1,440	45,600	66,240	7,144

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	50,232	77,440	250,000	464,640	500,000	—	—

Rat 15.—Piebald, weight 104 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	4	4,620	45,440

Day	8	9	10	11	—	—	—
Number of Trypanosomes per c.mm.	24,472	44,352	194,128	410,000	—	—	—

Rat 16.—White, weight 58 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	104	37,000	30,240
Temp.		42	44	36		44	46

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	126,000	86,640	121,600	189,000	160,800	—	—
Temp.	34	52	20	34	18	—	—

Note 'staircase' rise in the numbers of the parasites.

Rat 17.—White, weight 62 grams. Dose of inoculation = 38,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm.	—	—	—	—	1,500	500	1,380	4,02
Temp.		50	30	56	50			70

Note initial fall in numbers during first 24 hours.

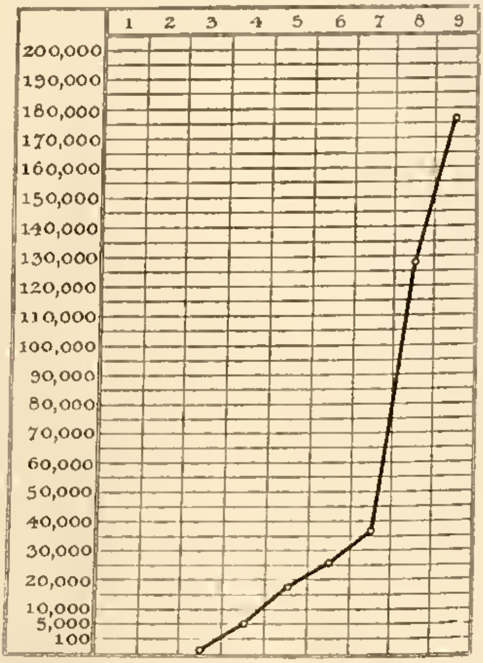

Chart 2.—Graph of Daily Counts of the Parasites in the Blood of a Rat inoculated with *T. rhodesiense*. Continuous rise. (See record of Rat 18.)

II.—RATS EXHIBITING CONTINUOUS RISE IN THE NUMBER OF PARASITES

Rat 18.—White ♂, weight 108 grams. Dose of inoculation: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	20	4,888	17,940	26,240	36,000
Temp.			34	42	50	48	30

Rat 18—continued.

Day	8	9	—	—	—	—	—
Parasites per c.mm.	129,024	175,200	—	—	—	—	—
Temp.		46	—	—	—	—	—

The graph of Rat 18 is represented in Chart 2.

Rat 19.—Piebald, weight 137 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	48	4,920	9,800	26,880
Temp.	40	40	52	62	50	62	52

Day	8	9	10	—	—	—	—
Parasites per c.mm.	31,000	51,080	160,000	—	—	—	—
Temp.	44	44	30	—	—	—	—

Rat 20.—Piebald ♂, weight 182 grams. Dose: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	20	4,352	6,640	7,776	20,088	126,000
Temp.	36	36	34	52	48	47	10	0

Rat 21.—Piebald ♂, weight 130 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	36	3,888	42,200	58,800
Temp.	38	36	38	50	34	58	20

Day	8	9	10	11	—	—	—
Parasites per c.mm.	86,832	97,200	112,000	144,000	—	—	—
Temp.	16	50	20	0	—	—	—

Rat 22.—White ♀, weight 125 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	244	18,000	66,560	81,000	176,80
Temp.	20	22	30	26	20	6	0

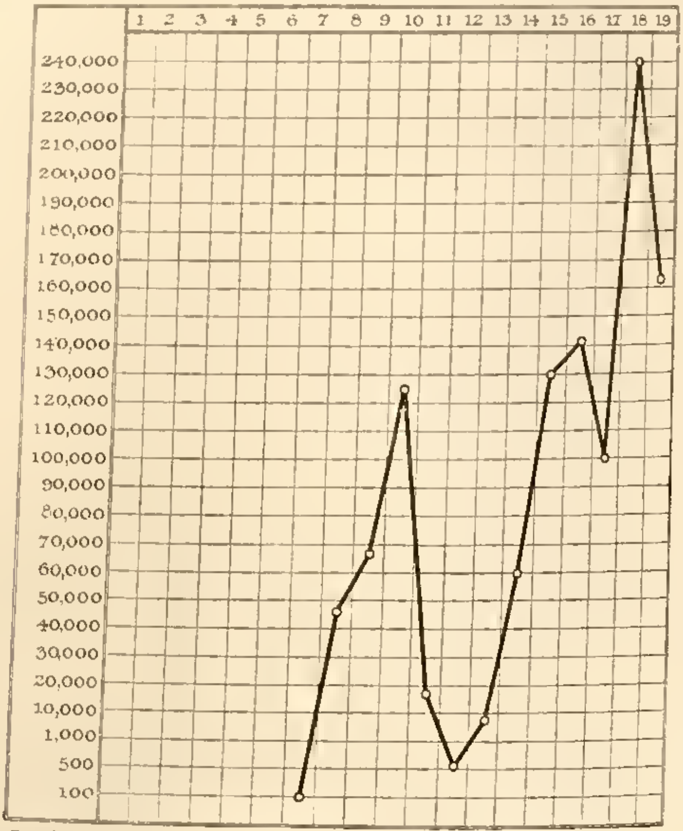

Chart 3.—Graph showing Daily Counts of the Parasites per c.mm. in the Peripheral Blood of a Rat inoculated with *T. gambiense*. Periodic Variation. (See record of Rat 23.)

(*b*) *Trypanosoma gambiense*

I.—RATS EXHIBITING PERIODIC VARIATION

Rat 23.—Piebald, weight 173 grams. Dose of inoculation: two million Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	120
Temp.	55	40	50	50	44	34	51

Rat 23—continued.

Day	8	9	10	11	12	13	14
Parasites per c.mm.	48,000	68,728	125,860	18,764	600	9,280	60,712
Temp.	45	65	50		50	66	38

Day	15	16	17	18	19	—	—
Parasites per c.mm.	130,000	142,000	100,000	240,000	164,000	—	—
Temp.	34	40	55		11	—	—

The graph of Rat 23 is represented in Chart 3.

RAT 24.—White ♂, weight 165 grams. Dose of inoculation: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—	4	6,000	1,920
Temp.		30	34	34	60	64	74	52

Day	9	10	11	12	13	14	15
Parasites per c.mm.	120	200	300	360	28,800	16,800	138,240
Temp.	54		20	54	54	58	10

RAT 25.—Piebald ♂, weight 155 grams. Dose: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	3,080	17,080	90,000	160,000
Temp.		50	22	10	44		66

Day	8	9	10	11	12	13	—
Parasites per c.mm.	19,100	7,800	7:800	10,080	76,800	38,800	—
Temp.	20	42	40	10	0	0	—

Rat 26.—White ♀, weight 150 grams. Dose: 150,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—	—	4	1,068
Temp.	48	55	46	65	60	62	58	54

Day	9	10	11	12	13	14	15
Parasites per c.mm.	4,892	768	1,680	2,860	9,688	6,384	2,436
Temp.	61	60	44	46	57	54	60

Rat 27.—White, weight 113 grams. Dose of inoculation: 60,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—	120	14,310	101,740
Temp.	61	49	32	12	44		5	40

Day	9	10	11	12	13	14	15
Parasites per c.mm.	146,880	69.400	23,276	55,224	67,096	57,552	6,120
Temp.	44	36	36	35			

Rat 28.—White, weight 101 grams. Dose of inoculation: 60,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	256	2,808
Temp.	59	70	43	42	40		56

Day	8	9	10	11	12	13	14
Parasites per c.mm.	4,416	3,648	24,904	90,560	118,700	143,520	270,000
Temp.	44	42	34	26	24		40

Rat 28—continued.

Day	15	16	17	—	—	—	—
Parasites per c.mm.	100,000	200,600	6.048	—	—	—	—
Temp.	78	42	—	—	—	—	—

Rat 29.—Piebald ♂, weight 230 grams. Dose: one million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	364	3,328	6,600	28,080	12,600
Temp.	36	40	56	60	47	82	56

Day	8	9	10	11	—	—	—
Parasites per c.mm.	2,808	16.380	19.360	150.000	—	—	—
Temp.	54	52	44	10	—	—	—

II.—RATS EXHIBITING CONTINUOUS RISE IN THE NUMBER OF PARASITES

Rat 30.—Piebald ♀, weight 125 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	—
Temp.	46	76	72		56	50	48

Day	8	9	10	11	12	13	14
Parasites per c.mm.	—	8	2,112	2,600	3,120	150,000	153,000
Temp.	62	65	42		34	58	26

Day	15	16	—	—	—	—	—
Parasites per c.mm.	181.440	540.000	—	—	—	—	—
Temp.	36	10	—	—	—	—	—

RAT 31.—Piebald ♂, weight 100 grams. Dose: 85,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	3,000	12,000	15,12
Temp.		34		30	34	32	46

Day	8	9	10	11	12	—	—
Parasites per c.mm.	24,000	101,400	200,600	378,560	216,000	—	—
Temp.	34	44	42	20	10	—	—

RAT 32.—Piebald ♀, weight 155 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	1,680	14,560	30,200	61,250	90,000
Temp.	34		34	66	52	50	44

Day	8	9	10	11	—	—	—
Parasites per c.mm.	220,500	409,600	504,000	360,000	—	—	—
Temp.	50	44	34	10	—	—	—

RAT 33.—White ♀, weight 40 grams. Dose: 100,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	32	8,100	48,150	88,000	126,000	180,000
Temp.			34		45		30	70

This rat was young. It is interesting to note that a count was made on the third day, five hours after the first count recorded in the table. There were then 544 parasites per c.mm., or $\frac{544}{32}$ = 17 times the first count, showing that four divisions of the parasite had taken place in five hours.

From these tables it will be seen that young and light rats, such as Numbers 18 and 33, are less resistant than older and heavier rats, e.g., Numbers 1 and 23.

It will also be seen that when the initial rise is rapid and high the rat soon dies, and does not show periodic variation.

COMPARISON OF THE TWO STRAINS IN RATS

We now append a table, for purposes of easy comparison, of various points regarding the two strains in rats. The figures are compiled from the foregoing data, and relate to enumerations in seventeen rats exhibiting periodic variation, and of five rats exhibiting continuous rise in the case of *T. rhodesiense*; also of seven rats exhibiting periodic variation and of four rats showing continuous rise in the case of *T. gambiense*.

(*a*) *T. rhodesiense*

No.	Colour	Weight in grams	Incubation period in days	Duration of life in days	No. of Trypanosomes inoculated	Approximate No. of Divisions in first 24 hours	Remarks
1	Piebald	187	2	14	1 million	3	(7 to 8 divisions in next 24 hours)
2	White	108	2	10	350,000	7	
3	White	225	1	9	2 million	8	
4	Piebald	220	3	13	500,000	8	
5	White	120	3	11	750,000	2	
6	Piebald	100	3	9	1 million	8	
7	Piebald	189	2	9	30,000	4	
8	White	195	3	13	100,000	Fall	(10 divisions in next 24 hours)
9	Piebald	100	3	12	500,000	8	
10	Piebald	150	3	15	500,000	8	
11	White	148	3	18	500,000	3	
12	White	138	4	12	600,000	8	Pregnant
13	White	140	4	16	200,000	4	Pregnant
14	Piebald	127	3	12	200,000	5	
15	Piebald	104	4	11	200,000	10	
16	White	58	4	12	200,000	8	
17	White	62	4	8	38,000	Fall	
18	White	108	2	9	2 million	8	See Chart 2
19	Piebald	137	3	10	1½ million	7	
20	Piebald	182	2	8	2 million	8	
21	Piebald	130	3	11	1½ million	7	
22	White	125	2	7	1½ million	6	
				Average ...	780,000		

(β) *T. gambiense*

No.	Colour	Weight in grams	Incubation period in days	Duration of life in days	No. of Trypanosomes inoculated	Approximate No. of Divisions in first 24 hours	Remark
23	Piebald	173	6	19	2 million	8	See Chart
24	White?	165	5	15	500,000	10	
25	Piebald	155	3	13	2 million	3	
26	White	150	6	15	150,000	8	
27	White	113	5	15	60,000	7	
28	White	101	5	17	60,000	3	
29	Piebald	230	2	11	1 million	3	
30	Piebald	125	8	16	500,000	8	
31	Piebald	100	4	12	85,000	2	
32	Piebald	155	2	11	1,500,000	3	Not very resistan
33	White	40	2	8	100,000	8	Young
				Average ...	723,000		

From the foregoing tables it follows:—

(1) That the average incubation period* in the case of *T. rhodesiense* was 2·9 days, whereas the average incubation period in the case of *T. gambiense* was 4·4 days.

(2) The average life of rats inoculated with *T. rhodesiense* was 11·3 days, whereas the life of rats infected with *T. gambiense* was 13·8 days.

These inferences are legitimate, for the average weight of the rats inoculated with *T. rhodesiense* was 138·8 grams, whereas the average weight of those inoculated with *T. gambiense* was 137 grams. Also the numbers of trypanosomes inoculated were nearly the same in the two strains, averaging 780,000 and 723,000 respectively.

(3) On comparing the number of divisions of parasites occurring in the first twenty-four hours in both strains, we find that it was approximately the same, namely, six divisions on the average, and varied in each case from two to ten divisions, most frequently being eight.

(4) There is also a shorter period between the crests of the waves in the graphs denoting the number of trypanosomes in

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* By incubation period we mean the interval (in days) from the moment of inoculation of the parasites into the host till the time the trypanosomes are first found in one cubic millimetre of the peripheral blood of host.

the Rhodesian strain (about three to four days) than in the laboratory strain of *T. gambiense* (viz., four to six days). (See Charts 1 and 3.)

(5) The difference in morphology between *T. rhodesiense* and *T. gambiense* has already been noted by Stephens and Fantham (1910).

(6) It follows, especially from the incubation period and the duration of life in sub-inoculated rats, that the Rhodesian strain is the more virulent. The difference of virulence is more markedly shown in the duration of life of infected guinea-pigs (see p. 457).

Curiously enough, we find that the incubation period does not seem to be affected so much by the dose (number of trypanosomes) inoculated as by the resistance of the animals. (Cf. Professor Ross's 'Prevention of Malaria' (1910), pp. 94, 95.)

ENUMERATIONS IN GUINEA-PIGS

Exactly the same methods as already set forth were followed in the counts of parasites in guinea-pigs. The same two strains of trypanosomes were again used, five animals being inoculated with each strain. We append tables of our results, together with typical charts.

1. *T. rhodesiense*

GUINEA-PIG 1.—Weight 346 grams. Dose of inoculation: 500,000 Trypanosomes. Incubation period 6 days. Duration of life 79 days. (J.G.T.)

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	4	28	44	20	8	48	2	4
Leucocytes	—	6,000	7,680	—	13,000	6,720	3,884	18,944	11,328	13,440	4,920	26,400	10,780	9,408
Temp.	—	80	84	76	—	70	76	75	74	70	78	—	74	80
Weight in grams	—	346	—	—	—	—	—	—	—	—	—	—	—	—

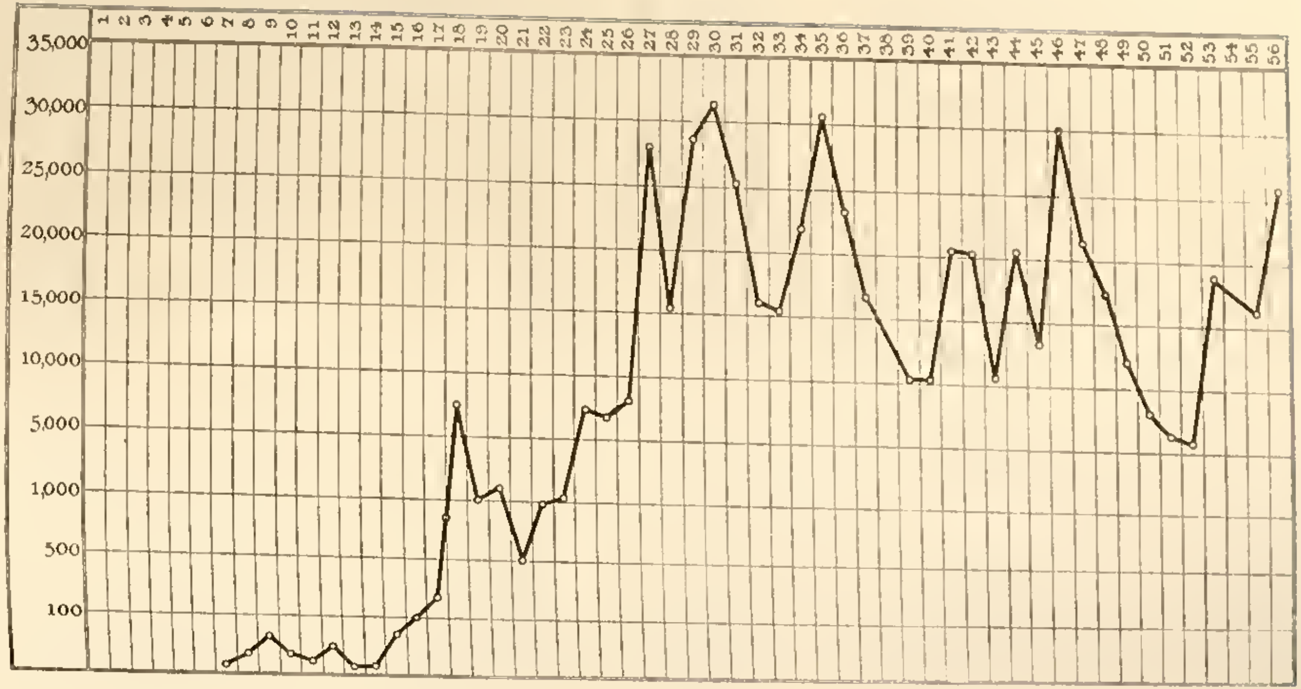

Chart 4.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. rhodesiense*. Periodic Variation. (See record of Guinea-pig 1.)

Guinea-pig 1—continued.

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	68	104	216	8,712	1,010	1,936	504
Leucocytes	—	—	—	—	—	—	—
Temp.	80	80	80	66	—	76	80
Weight in grams	—	408	—	—	—	—	—

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	1,152	1,360	8,576	8,064	9,936	28,224	15,504
Leucocytes	—	4,200	24,384	14,400	24,360	—	—
Temp.	76	76	74	76	—	80	81
Weight in grams	—	—	—	—	—	—	—

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	29,488	32,320	25,200	16,800	15,792	23,200	30,944
Leucocytes	—	—	—	—	—	—	—
Temp.	80	82	80	80	—	—	94
Weight in grams	—	425	—	479	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	23,688	16,800	—	10,560	10,520	21,280	20,208
Leucocytes	—	—	—	—	—	—	—
Temp.	81	83	—	—	—	82	—
Weight in grams	—	—	—	—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	11,172	21,334	14,784	30,448	24,812	17,952	12,960
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 1—continued.

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	9,920	8,920	7,448	19,824	—	17,612	26,496
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

The animal lived for 23 days after the last count was made.

The graph of Guinea-pig 1 is shown in Chart 4.

GUINEA-PIG 2, ♂.—Weight 880 grams when inoculated; 614 grams at death. Dose of inoculation: 500,000 Trypanosomes. Duration of life 28 days. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	2	0	1	4	4	12
Temp.		12	70	66	26		65	62

Day	9	10	11	12	13	14	15
Parasites per c.mm.	8	100	150	1,000	896	1,680	7,992
Temp.	74	70	64	66		76	68

Day	16	17	18	19	20	21	22
Parasites per c.mm.	6,000	5,632	7,200	21,600	45,600	25,000	11,340
Temp.	63	75	80	70	73	52	20

Day	23	24	25	26	27	28	
Parasites per c.mm.	6,720	4,416	18,720	9,600	6,000	30,720	Died
Temp.	80	82	10	64	78	6	

Guinea-pig 3, ♂.—Weight 952 grams at time of inoculation; 880 grams at death. Dose of inoculation: two million Trypanosomes. Duration of life 82 days. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	8	4
Temp.		25				20	

Day			46	47	48	49	50
Parasites per c.mm.	Interval of 38 days' vacation		12.600	80,064	68,448	23,562	9,972
Temp.			32	44	40	38	34

Day	51	52	53	54	55	56	57
Parasites per c.mm.	4,840	2,320	6.040	7.200	3,600	2,560	4,284
Temp.		44	30	40	68	26	28

Day	58	59	60	61	62	63	64
Parasites per c.mm.	8,600	12,480	23,296	3.328	4.320	1,600	3.072
Temp.		40	40	41	34	30	34

Day	65	66	67	68	69	70	71
Parasites per c.mm.	6,400	8,640	1,280	2.080	1,024	768	1,200
Temp.		46	30	44	40	42	43

Day	72	73	74	75	76	77	78
Parasites per c.mm.	10,560	6,048	5.880	2,480	4,320	5,760	4,032
Temp.		38	40	32	32	33	26

Guinea-pig 3—continued.

Day	79	80	81	82		—	—
Parasites per c.mm.	1,296	720	140	6,000	Died	—	—
Temp.	24	40	36	14		—	—

Guinea-pig 4, ♂.—Weight 440 grams at time of inoculation; 402 grams at time of death. Dose of inoculation: two million Trypanosomes. Duration of life 57 days. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	1,920	8	8	[illegible]
Temp.				13	22	31	22	33

Day		47	48	49	50	51
Parasites per c.mm.	Interval of 38 days' vacation	10,000	67,392	113,568	33,880	96,10
Temp.		20	24	46	32	

Day	52	53	54	55	56	57	
Parasites per c.mm.	50,000	26,740	14,864	7,168	3,024	24,720	Died
Temp.	40	36	36	49	34	2	

Guinea-pig 5, ♀.—Weight 492 grams when inoculated; 447 grams at death. Dose of inoculation: 1½ million Trypanosomes. Duration of life 51 days. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	—
Temp.			30				

Day	8	9	10	11	12	13	14
Parasites per c.mm.	—	—	—	12	4	56	40
Temp.	44	40	48	48	46	40	

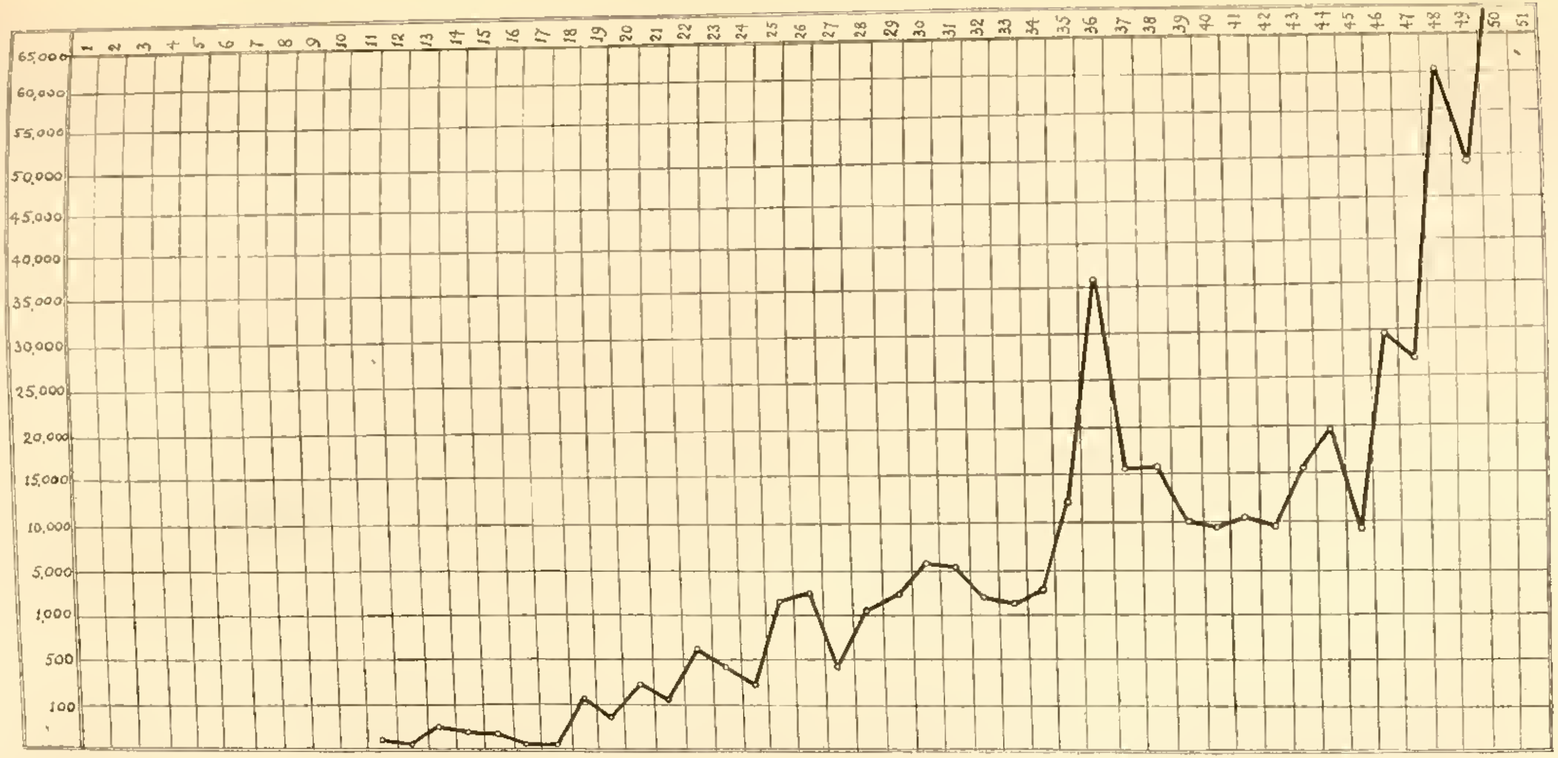

Chart 5.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. rhodesiense*. Periodic Variation. (See record of Guinea-pig 5.)

Guinea-pig 5—continued.

Day	15	16	17	18	19	20	21
Parasites per c.mm.	36	4	4	148	76	240	120
Temp.	36	52	30	46	34	30	24

Day	22	23	24	25	26	27	28
Parasites per c.mm.	660	420	260	2,700	3.264	432	1,024
Temp.	40	26	12	12	25	26	

Day	29	30	31	32	33	34	35
Parasites per c.mm.	3.200	6,400	5.440	2,744	2,352	3,520	12,800
Temp.	23	38	24	38	45	20	

Day	36	37	38	39	40	41	42
Parasites per c.mm.	35.200	15,680	15.760	10,000	9,408	10,240	9,800
Temp.	50	47	38	40	40	36	

Day	43	44	45	46	47	48	49
Parasites per c.mm.	15,120	19,600	9.600	29.568	27,320	60,624	49,140
Temp.	50	44	23	30	28	54	50

Day	50	51					
Parasites per c.mm.	96,880	164,160	Died	—	—	—	—
Temp.	38	0		—	—	—	—

The graph of Guinea-pig 5 is represented in Chart 5.

2. *T. gambiense*

GUINEA-PIG 6.—Weight 403 grams. Dose of inoculation: 4,000,000 Trypanosomes. Duration of life 79 days. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	12	16	24	8	0
Leucocytes	—	—	—	—	—	—	—
Temp.	78	73	91	82	84	70	77
Weight in grams	403	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	8	8	4	0	8	76	48
Leucocytes	—	—	—	—	—	—	—
Temp.	76	85	86	—	90	80	91
Weight in grams	—	—	—	—	—	—	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	680	1,440	960	—	272	440	416
Leucocytes	—	—	—	—	—	—	—
Temp.	80	76	80	—	74	76	88
Weight in grams	405	—	—	—	—	—	—

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	1,296	420	480	270	2,552	1,320	6,000
Leucocytes	—	—	—	—	—	—	—
Temp.	81	90	82	—	—	72	80
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 6—continued.

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	13.392	21,620	29,160	44.016	28,836	14.812	13,396
Leucocytes	—	—	13,428	—	16,200	7,452	7,548
Temp.	74	—	86	—	84	80	80
Weight in grams	427	—	—	—	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	29.592	39.720	52,600	39.396	27,840	67,032	113,160
Leucocytes	8,856	11,200	12,400	5,964	—	7,068	—
Temp.	70	76	70	—	82	76	82
Weight in grams	—	—	—	—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	88,000	100,400	45,080	110,400	60,904	40,240	55,240
Leucocytes	6,920	8,320	5,100	14,812	6,888	6,240	7,920
Temp.	82	82	80	—	70	78	80
Weight in grams	538	—	—	—	—	—	—

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	32,544	99,000	71,680	75.674	74,784	74,112	61,600
Leucocytes	4,032	8,530	—	—	—	—	—
Temp.	81	84	86	—	86	78	78
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 6—continued.

Day	57	58	59	60	61	62	63
Number of Trypanosomes per c.mm.	67,926	85,184	26,460	56,280	33,600	70.752	80,726
Leucocytes	—	—	9,976	—	—	—	—
Temp.	76	76	78	—	—	99	80
Weight in grams	574	—	—	—	—	—	—

Day	64	65	66	67	68	69	70
Number of Trypanosomes per c.mm.	64,000	50,232	27,456	44,000	63,962	91,056	72,432
Leucocytes	—	—	—	—	—	19,152	—
Temp.	80	—	—	—	76	—	—
Weight in grams	610	—	—	—	—	—	—

Day	71	72	73	74	75	76	77
Number of Trypanosomes per c.mm.	57,624	62.800	72.384	100,128	62,496	75,520	49,856
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Day	78	79	—	—	—	—	—
Number of Trypanosomes per c.mm.	80,808	53,176	Died	—	—	—	—
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 7.—Incubation period 4 days. Duration of life 105 days. (J.G.T.)

Day	20	21	22	23	24	25	26
Number of Trypanosomes per c.mm.	30,190	15.120	6,784	4,280	3,852	30,400	42,008
Temp.	—	86	80	76	83	89	92
Weight in grams	—	—	—	534	—	—	—
Leucocytes	—	—	—	—	—	—	12,000

Day	27	28	29	30	31	32	33
Number of Trypanosomes per c.mm.	32.212	28,120	7,914	18,698	13,860	23,440	21,720
Temp.	86	80	85	84	82	85	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	34	35	36	37	38	39	40
Number of Trypanosomes per c.mm.	8,040	13,784	8,460	9.720	9,600	32,572	—
Temp.	84	107	90	80	76	78	—
Weight in grams	—	—	—	467	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	41	42	43	44	45	46	47
Number of Trypanosomes per c.mm.	16,992	10,000	3,840	432	1,760	1,940	320
Temp.	82	80	82	84	84	86	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

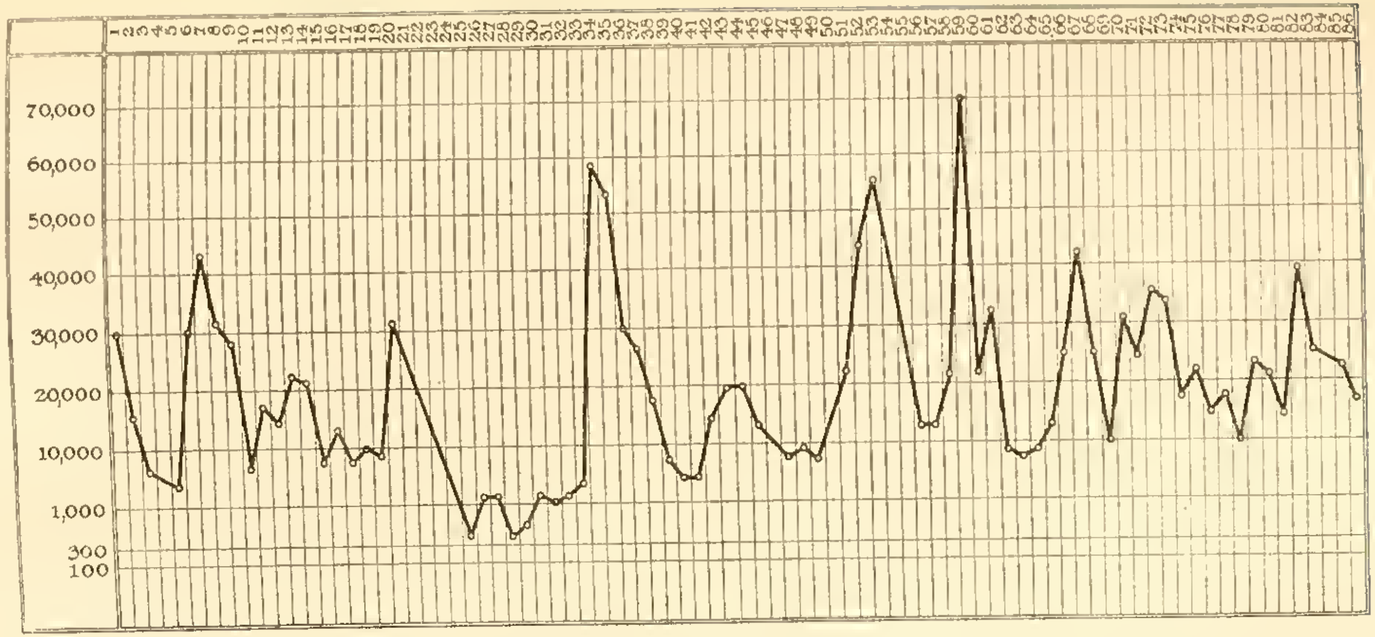

Chart 6.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. gambiense*. Periodic Variation. (See record of Guinea-pig 7.)

[The graph represents the daily numbers of the parasites from the 20th to the 105th day, when death occurred. The days should have been numbered accordingly, and not as 1 to 86.]

Guinea-pig 7—continued.

Day	48	49	50	51	52	53	54
Number of Trypanosomes per c.mm.	552	1,440	1,000	1,232	3,484	58,608	52,384
Temp.	—	78	76	76	—	72	—
Weight in grams	—	—	—	463	—	—	—
Leucocytes	—	—	—	—	—	17,160	26,784

Day	55	56	57	58	59	60	61
Number of Trypanosomes per c.mm.	31,124	26,416	18,368	8,112	5,840	5,184	15,656
Temp.	80	78	75	74	74	76	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	10,408	12,480	24,832	23,972	15,920	31,644	22,116

Day	62	63	64	65	66	67	68
Number of Trypanosomes per c.mm.	19,244	19,584	13,760	11,360	8,640	9,502	7,776
Temp.	73	78	80	80	80	70	76
Weight in grams	—	—	—	—	—	—	—
Leucocytes	18,564	18,432	—	26,960	20,480	41,272	—

Day	69	70	71	72	73	74	75
Number of Trypanosomes per c.mm.	15,800	23,848	42,480	57,240	45,800	30,120	12,144
Temp.	77	80	80	86	84	80	80
Weight in grams	—	—	—	500	—	—	—
Leucocytes (local)	—	12,672	25,040	15,480	14,560	—	—

Guinea-pig 7—continued.

Day	76	77	78	79	80	81	82
Number of Trypanosomes per c.mm.	12,180	21,328	70,000	21.160	32,944	9,048	7,392
Temp.	80	80	80	86	84	84	84
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	83	84	85	86	87	88	89
Number of Trypanosomes per c.mm.	9,056	12.760	25,632	42.000	25.000	10,570	31,680
Temp.	—	86	85	80	—	—	—
Weight in grams	—	—	—	513	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	90	91	92	93	94	95	96
Number of Trypanosomes per c.mm.	24,180	35,112	34,368	17,248	21,648	13,832	17,920
Temp.	82	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	97	98	99	100	101	102	103
Number of Trypanosomes per c.mm.	10,248	23,832	21,888	13,072	39,128	25,576	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	104	105	—	—	—	—	—
Number of Trypanosomes per c.mm.	23,056	17,848	Died	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

The graph of Guinea-pig 7 is represented in Chart 6.

GUINEA-PIG 8, ♂.—Weight 867 grams at time of inoculation; 830 grams on 92nd day; 755 grams at death. Dose of inoculation: 500,000 Trypanosomes. Duration of life 130 days. (H.B.F.)

Day	1	2	3	4	5	6	
Parasites per c.mm.	—	—	—	—	8	4	Interval of 38 days' vacation
Temp.		30			35	36	

Day	45	46	47	48	49	50	51
Parasites per c.mm.	7,540	8,640	5,808	13,156	14,400	18,000	1,280
Temp.	26	16	20	44	20	55	20

Day	52	53	54	55	56	57	58
Parasites per c.mm.	4,896	1.792	576	11,000	9,600	12,800	18,200
Temp.	34	46	30	33	38	50	46

Day	59	60	61	62	63	64	65
Parasites per c.mm.	9,216	1.024	2,916	6,000	7,680	10,000	2,000
Temp.	36	54	36	20	34	60	40

Day	66	67	68	69	70	71	72
Parasites per c.mm.	1.220	1,440	720	1,624	2.160	5,600	11,520
Temp.	30	46	26	50	52	26	64

Day	73	74	75	76	77	78	79
Parasites per c.mm.	6,624	4,480	5,760	3,600	5,760	2.016	2,400
Temp.	34	40	50	46	54	30	60

Guinea-pig 8—continued.

Day	80	81	82	83	84	85	86
Parasites per c.mm.	6.600	5,600	8.400	12,000	4,032	1,920	2,304
Temp.	20	24	42	36	46	76	72

Day	87	88	89	90	91	92	93
Parasites per c.mm.	2,184	2.160	3.920	7,840	4,608	3,360	5.040
Temp.	68	69	66	64	64	68	70

Day	94	95	96	97	98	99	100
Parasites per c.mm.	3,920	4,200	6.400	5,960	2,268	2,160	5,760
Temp.	84	64	70	62	60	64	62

Day	101	102	103	104	105	106	107
Parasites per c.mm.	15,680	19,584	9,792	18.000	36,960	5.400	9,600
Temp.	58	56	54	70	74	72	66

Day	108	109	110	111	112	113	114
Parasites per c.mm.	18,000	17,920	14,400	16,000	19,600	8.640	15.680
Temp.	72	74	66	70	76	72	80

Day	115	116	117	118	119	120	121
Parasites per c.mm.	23,040	5,600	9,600	10,240	6,400	12,800	10,240
Temp.	64	74	70	56	76	60	70

Guinea-pig 8—continued.

Day	122	123	124	125	126	127	128
Parasites per c.mm.	16,800	9.600	16,364	25,088	864	7,200	576
Temp.	70	80	74	88	74	82	74

Day	129	130	—	—	—	—	—
Parasites per c.mm.	900	15.000	Died	—	—	—	—
Temp.	86	0		—	—	—	—

GUINEA-PIG 9, ♀.—Weight 529 grams at time of inoculation; 440 grams at death. Dose of inoculation: 100,000 Trypanosomes. Duration of life 96 days. (H.B.F.)

Day	1	2	3	4	5	6	
Parasites per c.mm.	—	—	—	—	4	8	Interval of 38 days' vacation
Temp.		30			24	35	

Day	45	46	47	48	49	50	51
Parasites per c.mm.	28	36	84	72	228	240	256
Temp.	32	20	31	24	22		25

Day	52	53	54	55	56	57	58
Parasites per c.mm.	396	1,820	720	1,350	7,500	7,840	10,920
Temp.	30	20	20	28	38	20	25

Day	59	60	61	62	63	64	65
Parasites per c.mm.	15,340	10,368	7.680	8,064	15,680	16,800	18,400
Temp.	41	26	38	32	32	12	40

Guinea-pig 9—continued.

Day	66	67	68	69	70	71	72
Parasites per c.mm.	3,864	7,920	3,600	1,700	2,880	5,840	8,400
Temp.	30	35	30	25	30		44

Day	73	74	75	76	77	78	79
Parasites per c.mm.	12,800	9,600	7,200	3,000	8,640	9,200	8,400
Temp.	40	30	28	40	34	36	40

Day	80	81	82	83	84	85	86
Parasites per c.mm.	67,648	59,860	36,800	27,000	10,080	5,880	3,072
Temp.	30	20	26	42	20	70	74

Day	87	88	89	90	91	92	93
Parasites per c.mm.	5,120	7,840	3,600	6,272	5,400	6,720	25,600
Temp.	72	70	44	56	62	70	5

Day	94	95	96	—	—	—	—
Parasites per c.mm.	14,400	11,200	42,000	Died	—	—	—
Temp.	26	30	0	—	—	—	—

Guinea-pig 10, ♀.—Weight 495 grams at time of inoculation; 402 grams on 100th day; 335 grams at death. Dose of inoculation: two million Trypanosomes. Duration of life 147 days. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	—
Temp.	50		45			50	

Guinea-pig 10—continued.

Day	8	9	10	11	12	13	14
Parasites per c.mm.	4	0	0	52	4	4	16
Temp.	56	26	46	50	40	50	

Day	15	16	17	18	19	20	21
Parasites per c.mm.	200	16	4	4	4	12	8
Temp.	34	50	40	31	26	13	22

Day	22	23	24	25	26	27	28
Parasites per c.mm.	4	56	8	4	4	24	4
Temp.	23	44	20	24	28		20

Day	29	30	31	32	33	34	35
Parasites per c.mm.	4	4	8	4	20	20	8
Temp.	27	18	50	51	20	53	40

Day	36	37	38	39	40	41	42
Parasites per c.mm.	4	4	16	8	76	8	8
Temp.	36	36	31	34		26	34

Day	43	44	45	46	47	48	49
Parasites per c.mm.	0	0	0	0	24	20	480
Temp.	34	30	38	34	32	26	

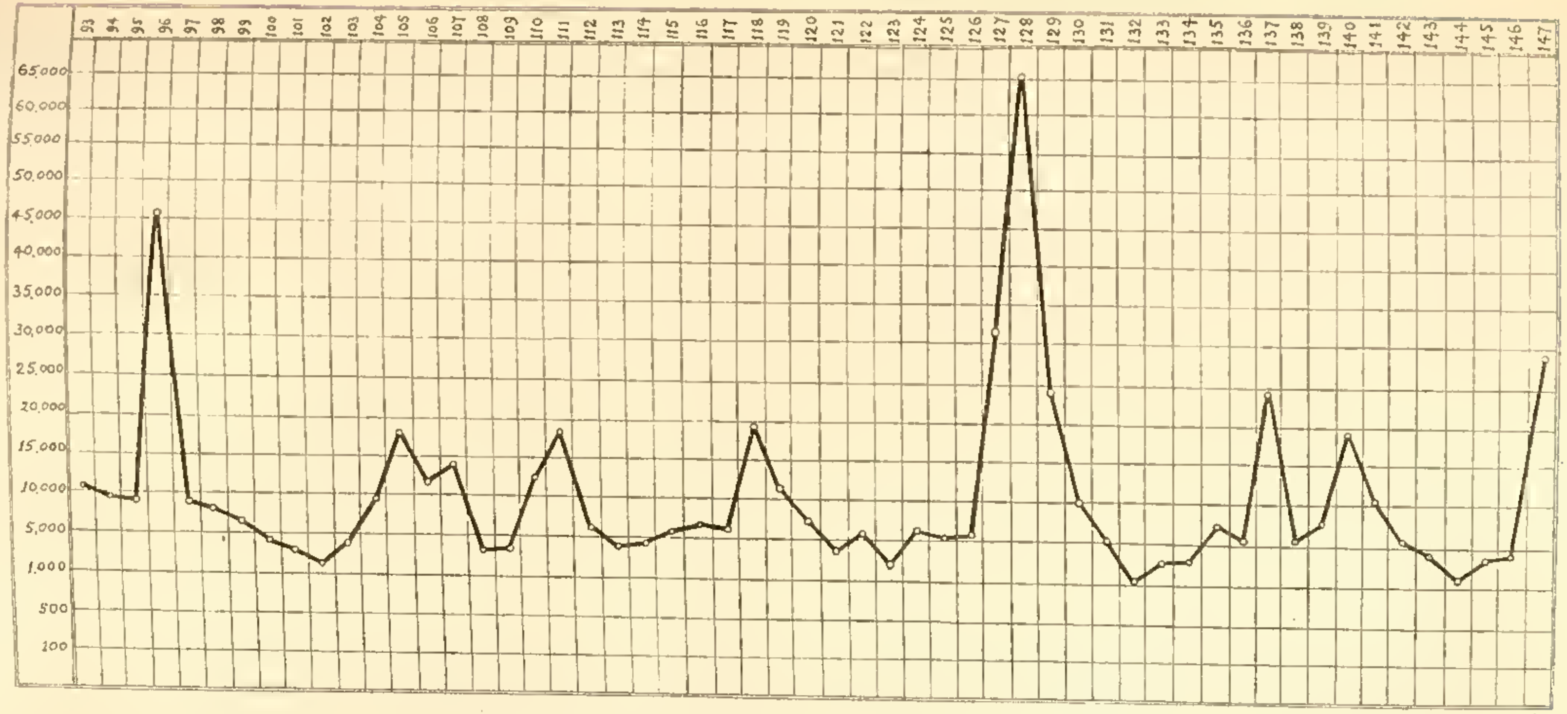

Chart 7.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. gambiense*. **Periodic Variation.** (See record of Guinea-pig 10.)

Guinea-pig 10—continued.

Day	50	51	52	53	54	
Parasites per c.mm.	1,880	416	124	368	116	Interval of 38 days' vacation
Temp.	40	34	23	32	15	

Day	93	94	95	96	97	98	99
Parasites per c.mm.	11,240	9,216	8,064	46,180	8,096	7,920	6,192
Temp.	34	44	30	32		34	42

Day	100	101	102	103	104	105	106
Parasites per c.mm.	4,864	3,300	2,000	4,576	9,240	18,200	12,480
Temp.	34	46	30	32		51	31

Day	107	108	109	110	111	112	113
Parasites per c.mm.	14,400	3,168	3,840	12,800	18,600	6,160	3,920
Temp.	20	50	42		60	50	32

Day	114	115	116	117	118	119	120
Parasites per c.mm.	4,536	5,040	6,912	6,000	19,200	12,000	7,020
Temp.	50	32	25		48	35	22

Day	121	122	123	124	125	126	127
Parasites per c.mm.	3,520	5,600	2,688	6,400	5,040	5,728	32,000
Temp.	40	30	44	38	36	40	40

Guinea-pig 10—continued.

Day	128	129	130	131	132	133	134
Parasites per c.mm.	66,000	24.500	10,000	5,040	1,008	3,600	3,888
Temp.	30	22	40	33	54	44	63

Day	135	136	137	138	139	140	141
Parasites per c.mm.	7,680	5,760	24,000	5,760	7,680	19,440	11,880
Temp.	44	70	44	70	78	72	96

Day	142	143	144	145	146	147	—
Parasites per c.mm.	6,480	4,860	2,268	3,888	4,480	28,880	Died
Temp.	80	76	30	52	14	0	

The graph of Guinea-pig 10 is represented in Chart 7.

The incubation period in the case of guinea-pigs varies so much in both strains that it is impossible to draw any definite conclusions therefrom.

The duration of life of guinea-pigs inoculated with the Rhodesian strain was shorter than in those similarly inoculated with *T. gambiense* (old laboratory strain); thus, the average life of the guinea-pigs inoculated with *T. rhodesiense* was 59 days, while the life of guinea-pigs inoculated with *T. gambiense* averaged 111 days.

There is a tendency for the period between the crests of the waves in the graphs of guinea-pigs to be slightly greater in the case of *T. gambiense* than in the case of *T. rhodesiense*.

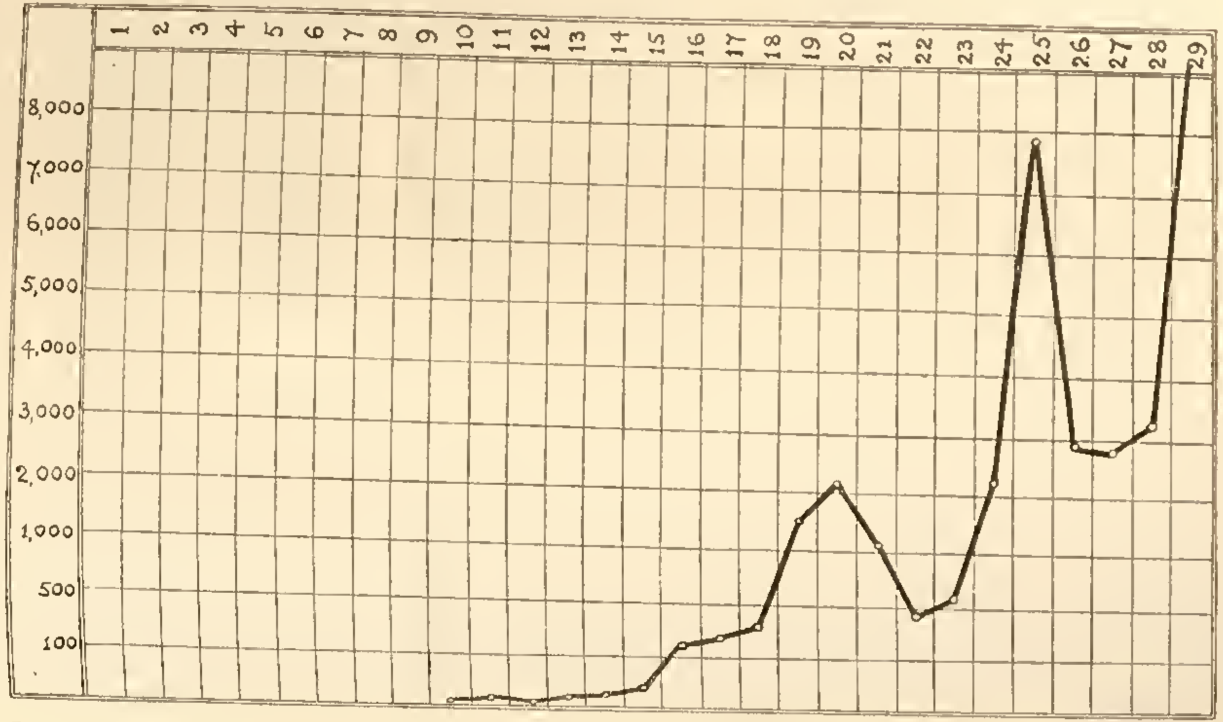

CHART 8.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Rabbit inoculated with *T. rhodesiense.* Periodic Variation. (See record of Rabbit 1.)

ENUMERATIONS IN RABBITS

Two rabbits were inoculated with *T. rhodesiense* and the course of the infection was followed in them, the same methods being employed as in previous cases. We append tables:—

T. rhodesiense

Rabbit 1.—Grey and white. Dose of inoculation: 600,000 Trypanosomes, intraperitoneally. (J.G.T.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	—
Day	8	9	10	11	12	13	14
Parasites per c.mm.	—	—	2	4	1	6	8
Day	15	16	17	18	19	20	21
Parasites per c.mm.	32	168	200	360	1,500	2,184	1,008
Day	22	23	24	25	26	27	28
Parasites per c.mm.	460	672	2,200	7,960	2,944	2,880	3,360
Day	29	—	—	—	—	—	—
Parasites per c.mm.	35,200	Died	—	—	—	—	—

The graph of Rabbit 1 is shown in Chart 8.

Rabbit 2.—Grey. Dose of inoculation: 600,000 Trypanosomes, intraperitoneally. (J.G.T.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	—
Day	8	9	10	11	12	13	14
Parasites per c.mm.	—	—	2	8	12	48	2
Day	15	16	17	18	19	20	21
Parasites per c.mm.	15	1	4	20	100	160	420
Day	22	23	24	—	—	—	—
Parasites per c.mm.	1,776	2,320	2,184	Died	—	—	—

COMPARISON OF THE EFFECTS OF THE TRYPANOSOMES ON THE VARIOUS HOSTS

In guinea-pigs the disease tended to run a more chronic course than in rats, as shown by the fact that the animals lived much longer. The periods between the crests of the waves in both strains in guinea-pigs were longer than in rats, namely five to eight days in most cases. Further, in guinea-pigs the initial rise is slower—a 'staircase' effect taking place and leading to the chronic condition (see Charts 4, 5).

Some of the guinea-pigs when the disease became chronic showed an alternation of high and low rises, which simulate remarkably the graph of the parasites of the patient W.A. (see R. Ross and D. Thomson (1910)*).

In the two rabbits used it was remarkable that these were the only animals that exhibited an affection of the eyes and skin. A white purulent discharge occurred from the eyes in both rabbits about the seventh day after inoculation. Simultaneously with this affection of the eyes the skin at the roots of the ears became encrusted with scabs. Both rabbits became drowsy early in the disease, and remained so until death. Although the incubation period in the case of rabbits was long (nine days), yet the animals early succumbed to the effects of the disease, in spite of the fact that the trypanosomes were never very numerous in the peripheral blood.

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* Proc. Roy. Soc., B, Vol. LXXXII, p. 413.

Rats also showed drowsiness during the latter half of the infection, while guinea-pigs continued to be lively and to take food well until a day or so before death, when in some cases drowsiness ensued.

In our experiments we did not observe any marked relation between the colour of the animal inoculated and the duration of infection in that animal. (It is sometimes thought that white rats are less resistant than piebald.)

In passing it may be noted, as has been done before, that the temperature of the host tended to rise with increase in the number of trypanosomes in the peripheral blood.

EXPLANATION OF THE PERIODIC INCREASE AND DECREASE IN THE NUMBERS OF TRYPANOSOMES IN THE PERIPHERAL BLOOD OF THE HOST

We believe that periodic increase and decrease of the numbers of trypanosomes in the peripheral blood of the host is a natural phenomenon, resulting from the reaction of the host and parasite on each other.

The parasites increase by longitudinal division until a maximum is reached when, unfavourable conditions arising, the formation of rounded, latent, non-flagellate bodies occurs, especially in the internal organs of the host where they collect in the spleen and bone marrow during the period of decrease of flagellates in the peripheral blood. These rounded bodies, or at least some of them, become flagellate trypanosomes when the parasites begin to increase in numbers again (see Fantham (1911)†).

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† Proc. Roy. Soc., B, Vol. LXXXIII, pp. 212-227. and Ann. Trop. Med. and Parasitol., Vol. IV. pp. 465-485.

The slight fluctuations in the periodicity, namely, the variations in the time-intervals between the crests of the waves, can be explained by the fact that the resistance of the animal varies from time to time. Again, we might note that the rat, the guinea-pig, and the rabbit are not the natural hosts of the parasites of sleeping sickness.

We should also note that the strains with which we have been dealing are very virulent, as judged by the records of Thomas and Linton (1904),* Moore and Breinl (1907), and others, whose inoculated animals lived much longer than ours. In less virulent strains the time-intervals between the crests of the waves in the graphs might tend to be longer.

The periodic variation or oscillation that we have described is of practical importance in the administration of drugs to eliminate trypanosomes in a host. If the drug be administered on the downward slope of the curve, the fall in numbers of trypanosomes is not due to the drug alone. But if the drug be administered on the upward slope of the curve, while the parasites are increasing in numbers, and yet a reduction in numbers of the parasites is brought about, then the drug may legitimately be inferred to be efficacious.

SUMMARY

1. In these investigations two strains of trypanosomes were used, namely, (*a*) *T. rhodesiense* (Stephens and Fantham) derived from a patient, W.A., suffering from sleeping sickness contracted in Rhodesia, and (*β*) *T. gambiense*, an old laboratory strain.

2. Of these two strains the Rhodesian was the more virulent, as judged by sub-inoculations in rats, guinea-pigs and rabbits, for the duration of life of animals inoculated with the Rhodesian strain was shorter than in those inoculated with *T. gambiense*.

Stephens and Fantham (1910) have already pointed out a distinct morphological peculiarity in *T. rhodesiense* in the presence of a posterior nucleus in some of the stout forms.

3. Enumeration of trypanosomes in the peripheral blood of rats, guinea-pigs and rabbits inoculated with each of these strains

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* Lancet, May 14, 1904, pp. 1337-1340.

reveals remarkable variations or oscillations in the numbers of the parasites from day to day, the variations being of the nature of a periodic increase and decrease at more or less regular intervals.

4. The periodicity is to be explained by (α) variations in resistance on the part of the host, probably due to the formation of anti-bodies, accompanied by (β) the formation on the part of the trypanosomes of rounded, latent, non-flagellate (relatively resistant) forms in the internal organs of the host during the fall in numbers of the flagellate parasites in the peripheral blood.

There is a life-cycle of trypanosomes in the Vertebrate host, in addition to stages of the parasite in the Invertebrate carrier (for example, *Glossina*).